Remarks

In accordance with the present invention, there are provided methods for testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ). Invention methods comprise assaying for changes in the level of reporter protein present as a result of contacting cells containing PPAR-γ (either endogenous to the host cell or introduced recombinantly) and a reporter vector with the compound of interest. Compounds identified employing invention methods are useful in the treatment of pathological conditions such as diabetes.

Claims 16-20, 27 and 28 were pending before this communication. By this communication, claim 16 has been amended and new claims 29-35 have been presented to define Applicants' invention with greater particularity. These amendments add no new matter and are fully supported by the specification and the original claims. Attached hereto is a marked-up version of the claims showing the changes made thereto, labeled APPENDIX A.

Accordingly, claims 16-20, 27 and 28-35 are currently pending. For the Examiner's convenience, a clean copy of all pending claims is provided in APPENDIX B.

Initially, Applicants acknowledge the withdrawal of the rejection of claims 16-20 and 27-28 under 35 U.S.C. § 112, second paragraph, in view of the previously submitted response (submitted July 26, 2002).

Applicants again traverse the rejection of claims 16-20, 27 and 28 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention (see Office Action mailed February 27, 2002, Paper No. 20).

Applicants respectfully disagree with the Examiner's attempts in the Advisory Action to limit the present claims to the situation where the PPAR-γ component used in the functional bioassay is a GAL4-PPAR-γ chimeric receptor, as shown in Example 3 (see specification at pages 23-24) (see Advisory Action, mailed August 8, 2002, Paper No. 26). Applicants respectfully submit that the claims of the present invention should not be limited to the specific working examples provided in the specification, because the specification enables multiple alternative embodiments of the claimed bioassay, as described in further detail below.

The present claims are directed to methods of testing compounds in a functional bioassay system. These methods require testing compounds using cells that contain (i) a PPAR-γ, and (ii) a reporter vector. Applicants have previously provided arguments demonstrating that the reporter vector component of the bioassay is fully enabled by the specification as filed (response submitted July 26, 2002). In the Advisory Action, the Examiner has for the first time raised new concerns regarding the enablement of the PPAR-γ component of the bioassay. Moreover, the Examiner erroneously asserts that the specification does not teach the claimed method when the host cell contains a non-chimeric PPAR-γ (in contrast to the GAL4-PPAR-γ chimeric receptor of Example 3). This attempt to limit the scope of the PPAR-γ component of the functional bioassay is clearly improper because the specification fully enables the claimed methods wherein (a) the PPAR-γ component of the bioassay is non-chimeric; and wherein (b) the PPAR-γ component of the bioassay is either introduced recombinantly or endogenous to the host cell.

(a) The specification fully enables the claimed methods wherein the PPAR-γ component of the bioassay is non-chimeric.

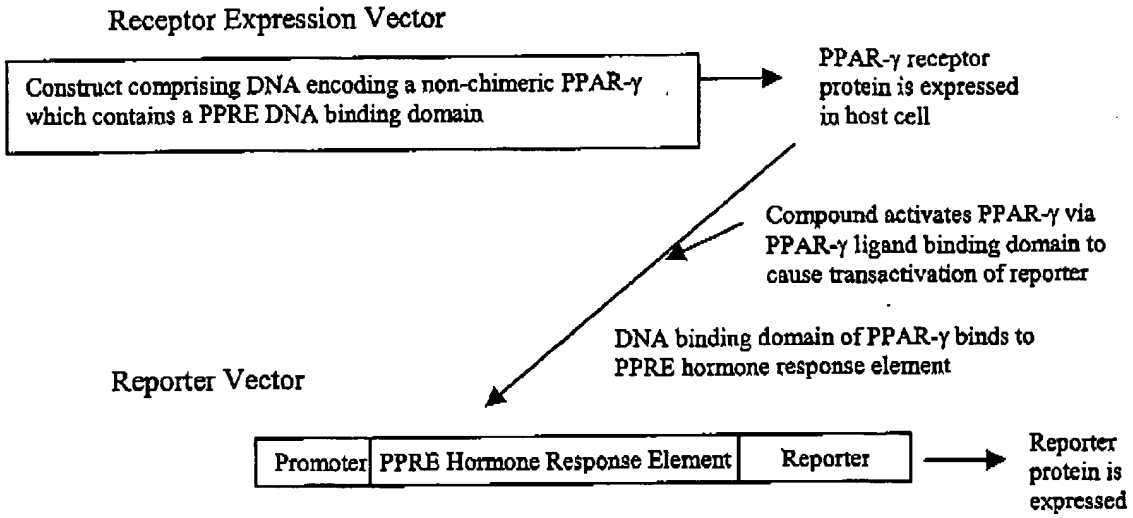
Applicants respectfully disagree with the Examiner's erroneous assertion that "[o]ne skilled in the art cannot predict that an increase or decrease in the level of reporter protein will be detected in the claimed assay since the receptor is not chimeric and does not contain a hormone response element binding domain (which is necessary to express the receptor protein)" (see Advisory Action, Paper No. 26, at lines 21-23). Contrary to the Examiner's assertion, the

specification clearly teaches that a non-chimeric PPAR-γ can be used in the functional bioassay, as is shown in the following exemplary section.

> Identification methods according to the present invention involve the use of a functional bioassay system, wherein the modified receptor [*i.e.*, a receptor expression plasmid in this example] and a reporter plasmid are cultured in suitable host cells in the present of test compound. . . .The expression plasmid can be any plasmid which contains and is capable of expressing DNA encoding the desired form of PPARγ receptor protein (i.e., **intact receptor or GAL4 chimeric receptor** as described hereinabove), in a suitable host cell.

(Emphasis added, see specification at page 15, lines 3-16). The complete nucleotide and amino acid sequence of an exemplary PPAR-γ is provided in SEQ ID NO:1. The hormone response element binding domain is the portion of the PPAR-γ protein that binds to DNA comprising the hormone response element, *e.g.*, a PPRE, as is well known in the art. The specification clearly describes exemplary hormone response elements contemplated for use as a portion of the reporter vector that are capable of being bound by an intact PPAR-γ such as that provided in SEQ ID NO:1 (see, for example, specification at page 13, line 13, through page 14, line 5).

Accordingly, one of skill in the art could readily assemble the components, for example, as depicted in the exemplary schematic below, to perform the claimed functional bioassay employing a non-chimeric PPAR-γ.



Applicants further disagree with the Examiner's unsupported assertion that a "large quantity of experimentation [is] necessary to regulate transcription activation of PPAR-gamma" and that "undue experimentation would be required of the skilled artisan to make and/or use the claimed invention" (see Advisory Action, Paper No. 26, lines 24-31). The specification clearly enables one of skill in the art to practice the claimed methods, whether the receptor employed is an intact receptor or part of a chimeric construct. The manipulations required to create vector constructs (as illustrated above), and to introduce them into host cells to perform the functional bioassay are routine molecular biological techniques. Additionally, the specification provides more than ample direction regarding both receptor and reporter components of the functional bioassay contemplated, in situations where the receptor is chimeric (as in Example 3) and where the receptor is not chimeric (as noted above). Thus, contrary to the Examiner's assertions in the Advisory Action, only routine experimentation is necessary to test compounds for their ability to regulate transcription-activating effects of PPAR-γ using the claimed assays. Therefore, Applicants have enabled the full scope of the claims as written.

(b) The specification fully enables the claimed methods wherein the PPAR-γ component of the bioassay is either introduced recombinantly or is endogenous to the host cell.

As illustrated above and clearly described in the specification, a PPAR-γ protein may be introduced into the host cells by an expression vector (see, for example, specification at page 15, lines 3-16). In performing the claimed functional bioassay with a PPAR-γ that is introduced recombinantly, the PPAR-γ expression plasmid and the reporter plasmid can be co-transfected into suitable host cells.

In efforts to advance prosecution and to further define this type of functional bioassay, claim 16 has been amended herein (pursuant to the Examiner's suggestion in the Advisory Action) to recite that the receptor component of the bioassay is introduced into the host cells by a receptor expression vector comprising a DNA segment encoding PPAR-γ. Claims 17-20, 27 and 28 are all dependent on claim 16.

However, Applicants respectfully submit that the specification also enables an alternative functional bioassay, wherein the PPAR-γ is endogenous to the host cell. For example, the specification teaches that "there is no need to use receptor-negative cells in carrying out the invention process", which allows for expression of native receptor by the test cell (see specification at page 17, lines 10-19). Thus, for example, the receptor component of the bioassay may be endogenously expressed by the host cell, and only the reporter component would need to be introduced recombinantly. Accordingly, new claims 29-35 are presented herein, which specifically recite that the host cells express native PPAR-γ. The methods of new claims 29-35 simply parallel the methods of claims 16-20, 27 and 28 in this alternative embodiment of the functional bioassay.

For all of the reasons cited above, it is respectfully submitted that the specification fully satisfies the requirements of 35 U.S.C. § 112, first paragraph. The scope of the claims is commensurate with the disclosure provided by the specification. It is clear that those skilled in the art would not require undue experimentation to practice the claimed invention. Accordingly,

reconsideration and withdrawal of the rejection of claims 16-20, 27 and 28 under 35 U.S.C. § 112, first paragraph, are respectfully requested.

Conclusion

In view of the above amendments and remarks, reconsideration and favorable action on all claims are respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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Enclosures: Appendices A and B

APPENDIX A – ALTERED CLAIMS

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

Claim 16 has been amended as follows:

16. (Twice amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding PPAR-γ, and

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein detected when said cells are contacted with said compound, relative to the level of the reporter protein detected when said cells are not contacted with said compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

New claims 29-35 have been added.

APPENDIX B – CURRENTLY PENDING CLAIMS

16. (Twice amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding PPAR-γ, and

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein detected when said cells are contacted with said compound, relative to the level of the reporter protein detected when said cells are not contacted with said compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

17. A method according to Claim 16 wherein said hormone response element is a direct repeat of two or more half sites separated by a spacer of one nucleotide, wherein said spacer can be A, C, G or T, wherein each half site comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-; and

wherein said response element is optionally preceded by N_x, wherein x falls in the range of 0 up to 5.

18. (Amended) A method according to claim 17 wherein said response element has at least one copy of the minimal sequence:

AGGACA A AGGTCA (SEQ. ID NO. 5),

wherein said minimal sequence is optionally flanked by additional residues.

19. (Amended) A method according to claim 17 wherein said response element has at least one copy of the sequence:

GGACC AGGACA A AGGTCA CGTTC (SEQ. ID NO. 6).

20. (Amended) A method according to claim 16 wherein said compound is a putative antagonist for said peroxisome proliferator activated receptor-gamma, and wherein said contacting is carried out in the presence of

increasing concentrations of said compound, and

a fixed concentration of at least one agonist for said peroxisome proliferator activated receptor-gamma,

wherein a decrease in the level of the reporter protein detected when said cells are contacted with said compound and said agonist, relative to the level of the reporter protein detected when said cells are contacted with said agonist alone, is indicative of a compound that is an antagonist of said receptor.

27. (Amended) A method according to Claim 16 wherein said contacting is carried out in the further presence of at least one PPAR-γ agonist,

wherein an increase or decrease in the level of the reporter protein detected when cells are contacted with said compound and said agonist, relative to the level of the reporter protein detected when cells are contacted with said agonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

28. (Amended) A method according to Claim 16 wherein said contacting is carried out in the further presence of at least one PPAR-γ antagonist,

wherein an increase or decrease in the level of the reporter protein detected when cells are contacted with said compound and said antagonist, relative to the level of the reporter protein detected when cells are contacted with said antagonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

29. (New) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said cells express native PPAR-γ, and

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein detected when said cells are contacted with said compound, relative to the level of the reporter protein

detected when said cells are not contacted with said compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

30. (New) A method according to claim 29, wherein said hormone response element is a direct repeat of two or more half sites separated by a spacer of one nucleotide, wherein said spacer can be A, C, G or T, wherein each half site comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-; and

wherein said response element is optionally preceded by N_x, wherein x falls in the range of 0 up to 5.

31. (New) A method according to claim 30, wherein said response element has at least one copy of the minimal sequence:

AGGACA A AGGTCA (SEQ. ID NO. 5),

wherein said minimal sequence is optionally flanked by additional residues.

32. (New) A method according to claim 30, wherein said response element has at least one copy of the sequence:

GGACC AGGACA A AGGTCA CGTTC (SEQ. ID NO. 6).

33. (New) A method according to claim 29, wherein said compound is a putative antagonist for said peroxisome proliferator activated receptor-gamma, and wherein said contacting is carried out in the presence of

increasing concentrations of said compound, and

a fixed concentration of at least one agonist for said peroxisome proliferator activated receptor-gamma,

wherein a decrease in the level of the reporter protein detected when said cells are contacted with said compound and said agonist, relative to the level of the reporter protein detected when said cells are contacted with said agonist alone, is indicative of a compound that is an antagonist of said receptor.

34. (New) A method according to claim 29, wherein said contacting is carried out in the further presence of at least one PPAR-γ agonist,

wherein an increase or decrease in the level of the reporter protein detected when cells are contacted with said compound and said agonist, relative to the level of the reporter protein detected when cells are contacted with said agonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

35. (New) A method according to claim 29, wherein said contacting is carried out in the further presence of at least one PPAR-γ antagonist,

wherein an increase or decrease in the level of the reporter protein detected when cells are contacted with said compound and said antagonist, relative to the level of the reporter protein detected when cells are contacted with said antagonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.